ORIGINAL RESEARCH

Assessment of Microbiological Classification, Clinical Features, and Outcomes in COVID-19-Associated Mucormycosis Patients: A Cross-Sectional Study

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ABSTRACT

Background: Mucormycosis is an uncommon invasive fungal disease. The present study was conducted to assess clinical features, and outcome in patients of COVID-19 associated Mucormycosis. **Materials & Methods:** 56 clinically suspected cases of mucormycosis of both genders were involved. Nasal secretions, nasal scrapings, biopsy specimens, and postoperative specimens. Each sample was inoculated onto three slants of Sabouraud dextrose agar and incubated for 14 days at a temperature of 30°C. Colonies exhibited rapid growth, densely covering the agar's surface with a cottony appearance. **Results:** There were 29 males and 27 females. Age group 20-30 years had 15, 30-40 years had 18, 40-50 years had 13 and >50 years had 10 patients. Place of residence was urban in 30 and rural in 26. The difference was non- significant (P> 0.05). Site involved was PNS only in 24, PNS and maxilla in 16, PNS and orbit in 5, PNS and intracranial in 6 and PNS and palate in 5 cases. KOH test was positive in 48 and negative in 7 cases. Culture was positive in 40 and negative in 16 cases. Genus was Rhizopus spp. in 42, Rhizomucour spp. in 10 and Lichtheimia spp. in 4 cases. Out of 56, 31 survived and 25 died. The difference was significant (P< 0.05). **Conclusion:** Males exhibited a higher prevalence of mucormycosis, which mainly impacted the paranasal sinuses. The common genus involved was Rhizopus spp. **Keywords:** COVID-19, Mucormycosis, Paranasal sinus, Rhizomucour

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Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

INTRODUCTION

Mucormycosis is an uncommon invasive fungal disease. This deadly illness gained attention during the SARS-CoV-2 pandemic, as patients recovering from or currently battling severe COVID-19 began to experience this invasive fungal infection as a post-COVID-19 complication, particularly in the second wave.¹ The illness is triggered by Mucormycetes from the Mucorales order and zygomycotic species.

Therefore, the illness is referred to as Zygomycosis as well.² The most prevalent clinical manifestation is the rhino-orbitalcerebral form, while other forms such as pulmonary, gastrointestinal, cutaneous, and disseminated are also present. The patients' prognosis is contingent on early treatment involving aggressive surgical debridement and systemic antifungals.³ Many factors can predispose COVID-19 patients to the development of mucormycosis. This encompasses uncontrolled diabetes mellitus, steroid-induced immunosuppression, extended stays in the Intensive Care Unit (ICU), posttransplant conditions/malignancies, voriconazole therapy, among others. Due to the fact that invasive mucormycosis has a very high mortality rate, it is extremely important to establish the correct diagnosis in a timely manner.⁴ Mucormycosis diagnosis relies primarily on clinical characteristics, which are often nonspecific, and examinations such as radiographic imaging that can identify organ involvement and disease extent.⁵ Only through fungal microscopy, fungal culture, and histopathology on appropriate biopsy samples can the definitive diagnosis and identification of the aetiological agent be established.⁶ The European Confederation of Medical Mycology (ECMM) advises in its "One World One Guideline" that when mucormycosis is suspected, suitable imaging to record the disease's extent is highly recommended.^{7,8}

AIM & OBJECTIVES

The present study was conducted to assess clinical features, and outcome in patients of COVID-19 associated Mucormycosis.

MATERIALS & METHODS

Study Design

This was a prospective observational study designed to assess clinical features and outcomes in patients diagnosed with COVID-19 associated mucormycosis.

Study Population

The study included 56 clinically suspected cases of mucormycosis. Participants were of both genders and had a recent history of COVID-19 infection or ongoing COVID-19 disease. All participants provided written informed consent.

Study Setting

The study was conducted in the Department of Microbiology in collaboration with Department of Pathology, ICARE Institute of Medical Sciences and Research and Dr. Bidhan Chandra Roy Hospital, Haldia (IIMSAR & BCRHH), West Bengal, India, with facilities for microbiological culture, histopathological diagnosis, and surgical management of mucormycosis.

Study Duration

The study was conducted over a period of one year from October 2020 to September 2021.

Inclusion Criteria

- Patients of any gender, aged ≥ 18 years.
- Clinically suspected mucormycosis based on symptoms like nasal congestion, facial

swelling, orbital pain, or black necrotic eschars.

- History of recent or ongoing COVID-19 infection (RT-PCR or antigen confirmed).
- Willingness to provide informed consent.

Exclusion Criteria

- Patients with other fungal infections (e.g., aspergillosis) without evidence of mucormycosis.
- Patients unwilling or unable to provide informed consent.
- Paediatric patients (<18 years of age).

Ethical Considerations

Approval for the study was obtained from the Institutional Ethics Committee prior to commencement. Written informed consent was obtained from all participants or their legal guardians. The study adhered to the principles of the Declaration of Helsinki.

Study Procedure

Clinical data were collected using a standardized proforma, which included:

- Demographic details (name, age, gender).
- History of comorbidities (e.g., Diabetes Mellitus).
- Use of systemic corticosteroids during COVID-19 treatment.
- Basal blood glucose levels on admission.
- Details of supportive oxygen therapy.
- Need for ICU admission.

Samples collected for microbiological evaluation included:

- Nasal secretions.
- Nasal scrapings.
- Biopsy specimens.
- Postoperative surgical specimens.

Each sample was inoculated on three slants of Sabouraud Dextrose Agar (SDA) and incubated at 30°C for 14 days. Growth characteristics, such as the appearance of rapid-growing, cottony white colonies, were recorded to confirm mucormycosis.

Surgical Technique

Patients diagnosed with mucormycosis underwent surgical debridement as per the extent of disease. Endoscopic sinus surgery, orbital exenteration, or maxillectomy were performed based on clinical judgment and radiological findings.

Outcome Measures

- Clinical outcome (recovery, morbidity, or mortality).
- Duration of hospital stay.
- Requirement of surgical intervention.

- Post-operative complications.
- Mortality rate within the study period.

Statistical Analysis

All data were entered into a structured database and subjected to statistical analysis using SPSS software version 20.0. Continuous variables were **RESULTS** expressed as mean \pm standard deviation, and categorical variables as frequencies and percentages. Associations were evaluated using Chi-square or Fisher's exact test where appropriate. A p-value < 0.05 was considered statistically significant.

Table 1: Baseline characteristics				
Parameters	Variables	Number	P value	
Gender	Male	29	0.95	
	Female	27		
Age group (years)	20-30	15	0.69	
	30-40	18		
	40-50	13		
	>50	10		
Residence	Urban	30	0.43	
	Rural	26		

Table 1 show that there were 29 males and 27 females. The p-value is 0.95, which suggests that there is no statistically significant difference in the gender distribution of participants. Age group 20-30 years had 15, 30-40 years had 18, 40-50 years had 13 and >50 years had 10 patients. The p-value for age group is 0.69, which also

indicates that there is no significant difference between the age groups in the study. Place of residence was urban in 30 and rural in 26. The pvalue for residence is 0.43, which again shows no significant difference between urban and rural participants.

Parameters	Variables	Number	P value
Site of Infection	PNS only	24	0.01
	PNS and maxilla	16	
	PNS and orbit	5	
	PNS and intracranial	6	
	PNS and palate	5	
KOH test	Positive	48	0.01
	Negative	7	
Culture	Positive	40	0.02
	Negative	16	
Genus identification	Rhizopus spp.	42	0.04
	Rhizomucour spp.	10	
	Lichtheimia spp.	4	
Outcome	Survived	31	0.03
	Died	25	

 Table 2: Assessment of clinical and microbiological findings along with patient outcomes

Table 2, figure I shows that site involved was PNS(Paranasal sinuses) only in 24, PNS and maxilla in 16, PNS and orbit in 5, PNS and intracranial in 6 and PNS and palate in 5 patients respectively. The most common site of infection was confined to the paranasal sinuses (PNS). Some patients had more extensive disease involving the maxilla, orbit, intracranial region, or palate, indicating progressive or severe forms of mucormycosis.

A p-value of 0.01 suggests that the differences in site involvement are statistically significant, implying that the disease can vary widely in terms of anatomical spread.

KOH test was positive in 48 and negative in 7 cases. The KOH mount is a rapid diagnostic test used to detect fungal elements. A high proportion of patients tested positive, supporting the test's value in early diagnosis. The significant p-value indicates that KOH positivity is a common and relevant finding in these patients.

Fungal cultures were positive in a majority of cases (40 patients), confirming the presence of mucormycosis-causing fungi and negative in 16 cases. The p-value of 0.02 signifies a statistically significant difference between culture-positive and culture-negative cases, highlighting the importance of culture in diagnosis and species identification.

Fungal Genus Identified was Rhizopus spp. in 42, Rhizomucour spp. in 10 and Lichtheimia spp. in 4 cases. Among the patients with a

positive culture, Rhizopus spp. was the most frequently isolated organism, consistent with global trends where it is the predominant causative agent of mucormycosis. The p-value of 0.04 shows a significant variation in the distribution of fungal species, suggesting that Rhizopus is significantly more common than other genera in this study.

Out of 56, 31 survived and 25 died. Nearly 45% of the patients died, reflecting the high mortality associated with COVID-19-associated mucormycosis. The difference was significant (P < 0.05).



PNS= paranasal sinuses

DISCUSSION

The pandemic of coronavirus disease 2019 (COVID-19), caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China, and soon spread globally.9,10 Due to the influence of various factors such as age, gender, co-morbidities, education status, and viral load on the severity of COVID-19, patients infected with SARS-CoV-2 exhibit a broad spectrum of clinical manifestations ranging from asymptomatic cases or mild upper respiratory symptoms to multiple organ failure (MOF) and death.^{11,12}

We found that there were 29 males and 27 females. Age group 20-30 years had 15, 30-40 years had 18, 40-50 years had 13 and >50 years had 10 patients. Place of residence was urban in 30 and rural in 26. Gupta et al¹³ described and characterised the genus of the causative agents of mucormycosis, an invasive fungal infection, and to analyse the clinical features and outcomes in

the study participants. The study variables included demographic factors, presence of risk factors, organ involvement, and in-hospital mortality. Diagnosis was based on fungal Potassium Hydroxide (KOH) mount and fungal culture. Genus characterisation was performed using Lactophenol Cotton Blue (LPCB) mount. The majority of patients with the disease were male (77, 74%). The most commonly involved site was isolated paranasal sinuses (48, 46.1%), followed by paranasal sinus with maxilla involvement (24, 23.1%). The KOH report was positive in 100 (96.2%) of patients. Fungal growth in culture was reported in 69 (66.3%) patients, among them Rhizopus spp. (66, 95.6%) being the most common fungal genus isolated. Among 25 patients in the age category >60 years, 21 (84%) had a positive culture (p-value=0.041, significant). Diabetic patients had a significantly higher incidence of mucormycosis compared to non- diabetics based on culture results (pvalue=0.004). The mortality rate in this study was 26 (25%).

We found that site involved was PNS only in 24, PNS and maxilla in 16, PNS and orbit in 5, PNS and intracranial in 6 and PNS and palate in 5 cases. KOH test was positive in 48 and negative in 7 cases. Culture was positive in 40 and negative in 16 cases. Genus was Rhizopus spp. in 42, Rhizomucour spp. in 10 and Lichtheimia spp. in 4 cases. Out of 56, 31 survived and 25 died. Gupta et al¹⁴ in their study a consortium of clinicians from various parts of India studied clinical profile of COVID-19 associated mucormycosis (CAM) was studied. Investigators from multiple sites in India were involved in this study. Clinical details included the treatment and severity of COVID-19, associated morbidities, as well as the diagnosis, treatment and prognosis of mucormycosis. These data were collected using google spreadsheet at one centre. Descriptive analysis was done. There were 115 patients with CAM. Importantly, all patients had received corticosteroids. Diabetes was present in 85.2% of patients and 13.9% of patients had newly detected diabetes. The most common site of was involvement rhino-orbital. Mortality occurred in 25 (21.7%) patients. On logistic regression analysis, CT scan-based score for severity of lung involvement was associated with mortality.

LIMITATIONS OF THE STUDY

- Sample Size: The study was limited to 56 patients, which may not be representative of the general population.
- Single-centre Study: Results may not be generalizable to different geographic or demographic populations.
- Lack of Long-term Follow-up: The study did not assess long-term outcomes or recurrence.
- Possible Reporting Bias: As data collection depended partly on patient-reported history (e.g., corticosteroid use), recall bias could not be ruled out.
- Unaccounted Confounders: Factors such as nutritional status, socioeconomic status, and access to healthcare were not considered in the analysis.

CONCLUSION

Authors found that males exhibited a higher prevalence of mucormycosis. COVID-19associated mucormycosis predominantly involved the paranasal sinuses, with Rhizopus spp. being the most common fungal isolate. KOH and culture were effective diagnostic tools. Despite timely diagnosis and treatment, the mortality rate was high (45%), emphasizing the need for early detection and aggressive management to improve outcomes.

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